



UNIVERSITI PUTRA MALAYSIA

**EFFECTS OF ENRICHED BIOCHAR AND *Bacillus subtilis* ON GROWTH,
PHYSIO-BIOCHEMICAL PROPERTIES AND FUSARIUM WILT
INCIDENCE OF BANANA UNDER WATER STRESS**

SITI NORLIZA MOHD DIN

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By

SITI NORLIZA BINTI MOHD DIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia
in Fulfillment of the Requirement for the Degree of Master of Science**

September 2018



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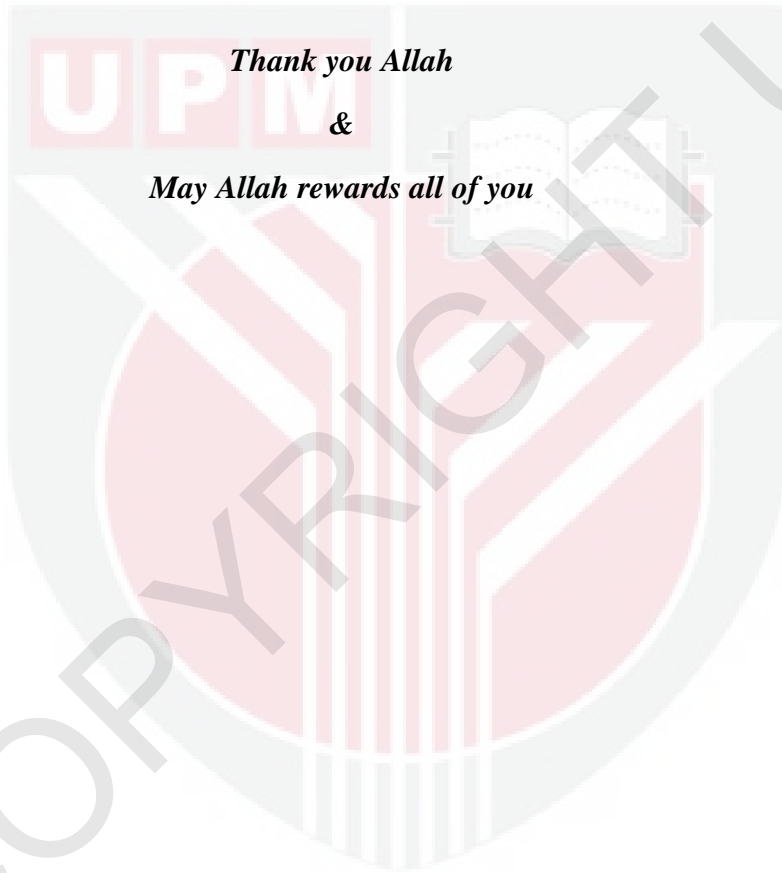
DEDICATION

This research study is dedicated to all scientists, researcher and lecturer around the world who are struggles to keep the sanctity of our earth's atmosphere preserved. For my great and very helpful supervisor Prof Madya Dr. Siti Zaharah Sakimin, the guidance and support you give throughout my study period is priceless. For my beloved parents, Mohd Din Bin Jusoh (father) and Rokiah binti Dollah (mother), this thesis is dedicated for both of you, the cost of bring me up until this level is incalculable.

Thank you Allah

&

May Allah rewards all of you



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

EFFECTS OF ENRICHED BIOCHAR AND *Bacillus subtilis* ON GROWTH, PHYSIO-BIOCHEMICAL PROPERTIES AND FUSARIUM WILT INCIDENCE OF BANANA UNDER WATER STRESS

By

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September 2018

Chairman : Siti Zaharah Sakimin, PhD
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Banana (*Musa acuminata* cv. Berangan) is one of the most popular fruit and has great potential for commercial development since the current demand had increased drastically. However, in Malaysia, poor soil fertility, limited water availability and disease attack are among common problems in banana industry. In order to find a solution of the problems, a field study was conducted to determine the optimum enriched biochar rate for improvement of soil physicochemical properties, growth and physiological status of banana. In addition to field study, a glasshouse study was conducted to determine the effect of *Bacillus subtilis* inoculation in enriched biochar media and water stress on growth, physiological status and suppression of Fusarium wilt. Four different rates of enriched biochar (0, 1.5, 3.0 and 4.5 t ha⁻¹) were applied once by mixing with Bungor Series soil and put into a polybag with the size of 40 cm × 40 cm. One month old banana plantlets were used. The treatments were carried out for 3 months and arranged in a randomized complete block design with 4 replications. Meanwhile, in the second study, the media were prepared based on optimum rate of enriched biochar from the first study. The treatments consisted of two factors (water stress and *Bacillus subtilis*) arranged by split plot in randomized complete block design with three replications. The media were enriched with 0, 20, 40 and 60 mL *Bacillus subtilis* at the concentration 10⁸ CFU mL⁻¹ applied by soil drenching as pre-inoculation treatments and plantlets were subjected to 100% well-watered (WW), 75% medium-stressed (MS) and 50% severe-stressed (SS) treatments based on field capacity (FC) level. The plantlets inoculated with *Fusarium oxysporum* race 4 (FOC) one week after *Bacillus subtilis* treatments were applied and the plant were destructively sampled at 45 and 90 days after transplanting (DAT). The results found that soil microbial population, soil physicochemical properties (pH, CEC, total C, N, K, Ca and Mg), growth characteristics (plant height, pseudo-stem diameter, total leaf number and total leaf area), mineral content in leaf tissue (N, P, K, Ca and Mg) and photosynthesis rate increased with the increasing rate of

enriched biochar and 4.5 t ha⁻¹ was selected as the best treatment. SS treatment significantly reduced growth parameters and physiological status (photosynthesis, stomatal conductance, transpiration and relative water content) and increased accumulation of proline and malondialdehyde content in plant tissue. Chlorophyll content showed increased by higher rates of *Bacillus subtilis* at both 45 and 90 DAT of destructive period. High concentrations of *Bacillus subtilis* (40 and 60 mL) also were found to reduce disease severity under SS but the value increased under MS and WW condition following the time. This finding suggested that, soil amendment with enriched biochar at 4.5 t ha⁻¹ was adequate for optimum growth at nursery level (vegetative stages up to 3 months). Inoculation of the media with higher rates of *Bacillus subtilis* able to improved growth, physiological status and nutritional properties of the banana plants under water stress condition, reduced the deleterious effects of stress and helped plantlets to tolerate drought stress to a higher level as compared to non-inoculated plantlets and increase resistance to Fusarium wilt.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN BIOCHAR DIPERKAYA DAN *Bacillus subtilis* TERHADAP
PERTUMBUHAN, SIFAT-SIFAT FISIO-BIOKIMIA DAN KEJADIAN LAYU
FUSARIUM POKOK PISANG DI BAWAH KEADAAN TEGASAN AIR**

Oleh

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Pisang (*Musa acuminata* kultivar Berangan) adalah salah satu buah-buahan yang paling popular dan mempunyai potensi yang tinggi untuk pembangunan komersial kerana permintaan semasa telah meningkat secara drastik. Walau bagaimanapun, di Malaysia, kesuburan tanah, bekalan air yang terhad dan serangan penyakit adalah antara masalah biasa dalam industri pisang. Untuk mencari penyelesaian kepada masalah tersebut, kajian lapangan telah dijalankan untuk menentukan kadar optimum biochar diperkaya untuk penambahbaikan kepada sifat-sifat fizikal kimia tanah, pertumbuhan dan status fisiologi pokok pisang. Tambahan kepada kajian lapangan, satu kajian dalam rumah kaca telah dijalankan bagi menentukan kesan inokulasi *Bacillus subtilis* dalam biochar diperkaya dan tegasan air terhadap pertumbuhan, status fisiologi dan pengurangan kepada penyakit layu fusarium. Empat kadar berbeza biochar diperkaya (0, 1.5, 4.5 dan 3.0 t ha⁻¹) dicampur sekali dengan tanah siri Bungor dan dimasukkan ke dalam polibeg saiz 40 cm × 40 cm. Anak pisang berusia sebulan digunakan. Kajian ini telah dijalankan selama 3 bulan dan disusun mengikut reka bentuk susunan blok rawak dengan 4 replikasi. Sementara itu, dalam kajian kedua, media disediakan berdasarkan kadar optimum biochar diperkaya dari kajian pertama. Rawatan terdiri daripada dua faktor (tegasan air dan *Bacillus subtilis*) diatur dengan reka bentuk split plot dalam susunan blok rawak dengan tiga replikasi. Media telah di inokulasi dengan kadar *Bacillus subtilis* berbeza iaitu 0, 20, 40 dan 60 mL pada kepekatan 10⁸ CFU mL⁻¹ pada tanah sebagai rawatan pre-inokulasi serta didedahkan dengan tiga aras tegasan air iaitu 100% tiada tegahan (WW), 75% tegahan sederhana (MS) dan 50% tegahan teruk (SS) berdasarkan kapasiti lapangan (FC). Anak pokok diinokulasikan dengan *Fusarium oxysporum* race 4 (FOC) seminggu selepas inokulasi *Bacillus subtilis* dan anak pokok di cabut pada 45 hari dan 90 hari selepas pemindahan. Keputusan mendapati populasi mikroba tanah, sifat fizikal kimia tanah (pH, CEC, jumlah C, N, K, Ca dan Mg), ciri-ciri

pertumbuhan (ketinggian pokok, diameter pseudo-stem, jumlah bilangan daun dan luas daun), kandungan mineral dalam daun (N, P, K, Ca dan Mg) dan fotosintesis meningkat dengan peningkatan kadar biochar diperkaya dan kadar 4.5 t ha⁻¹ dipilih sebagai kadar terbaik. Rawatan SS menunjukkan pengurangan yang ketara bagi parameter pertumbuhan, status fisiologi (fotosintesis, konduktan stomata, transpirasi dan kandungan air relatif) dan telah meningkatkan kandungan proline serta malondialdehyde dalam tisu tumbuhan. Kandungan klorofil juga meningkat dengan peningkatan kadar *Bacillus subtilis* pada kedua-dua tempoh anak pokok dicabut iaitu 45 dan 90 hari selepas pemindahan (DAT). Kepekatan yang tinggi *Bacillus subtilis* (40 dan 60 mL) juga telah didapati dapat mengurangkan insiden penyakit di bawah keadaan SS tetapi nilai tersebut meningkat di bawah keadaan MS dan WW mengikut peredaran masa. Kajian ini mendapati tanah campuran dengan biochar diperkaya pada kadar 4.5 t ha⁻¹ mencukupi untuk pertumbuhan optimum di peringkat nurseri (tahap vegetatif 1-3 bulan). Inokulasi media dengan *Bacillus subtilis* juga mampu memperbaiki pertumbuhan, status fisiologi dan ciri nutrisi pokok pisang di bawah keadaan tegahan air, mengurangkan kesan bahaya tekanan dan membantu anak pokok bertahan di bawah keadaan kekurangan air ke tahap yang lebih tinggi berbanding pokok yang tidak diinokulasi dan meningkatkan kerintangan pokok terhadap serangan penyakit layu fusarium.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AA	Auto Analyzer
AAS	Atomic Absorption Spectrometer
ABA	Absciscic Acid
CFU	Colony Forming Unit
CV	Coefficient of Variation
cv.	Cultivar
C	Carbon
Ca	Calcium
C:N	Carbon to Nitrogen Ratio
CEC	Cation Exchange Capacity
DAT	Day After Transplanting
DF	Degree of Freedom
DOA	Department of Agriculture
EC	Electrical Conductivity
EFB	Empty Fruit Bunch
et al.	And Friends
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistical Database
FC	Field Capacity
FW	Fresh Weight
gs	Stomata Conductance
K	Potassium
Kg/polybag	Kilogram per polybag
LSD	Least Significant Different
Ps	Photosynthesis
PGPR	Plant Growth Promoting Rhizobacteria
R:S	Root to shoot ratio
RCBD	Randomized Complete Block Design
rpm	Rotation Per Minute
RM	Ringgit Malaysia
SAS	Statistical Analysis System
Ts	Transpiration Rate
vs	Versus
v/v	Volume per volume
VPD	Vapour Pressure Deficit
WUE	Water Use Efficiency
WS	Water Stress



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CHAPTER 1

INTRODUCTION

Banana which is considered as “queen of tropical fruits” ranked fourth in term of production after oil palm, rubber and paddy. It is also the second most widely cultivated fruit crop which covers 10% of total fruit area (297, 860 ha) and total production of 535, 000 tonnes (Mohamad Roff, 2012). Production value of banana also had increased from RM3.63 billion in 2009 to RM4.57 billion in 2013 (Anonymous, 2014). Currently, major amount of banana production goes to local consumption and only remaining 15% are for export (Kayat et al., 2016). Most cultivated cultivars are Pisang Berangan, Cavendish and Pisang Tanduk which is commonly consumed as sweet dessert and Pisang Tanduk for crisp production. Other famous cultivated cultivar includes Pisang Nangka, Pisang Rastali, Pisang Mas, Pisang Abu and Pisang Awak (Kayat et al., 2016).

Since banana consumption is increasing every year, it has gained attention for the government to increase the cultivation area. The effort has been given by selecting certain area for banana cultivation. For instance, the district of Jeli has been chosen as main area and supplier of banana in Kelantan. The project was also implemented in other state in Malaysia.

Despite of a good government intention to increase banana production, there are some restriction encounters by banana growers to achieve optimum growth and high banana yield in Malaysia. Poor soil fertility, limited water availability and disease attack are among of the major problems reported recently. In Malaysia, 72% areas are covered by ultisol and oxisol. The soils are highly weathered, high accumulation of sesquioxides, prone to leaching of plant nutrients and lacking in organic matter (Anda et al., 2010). Besides that, soil in Malaysia is usually acidic as Malaysia receives high rate of total rain annually with pH between 4.2 to 4.8. Acidic rain water will drain directly into the soil surface and will destroy the mineral and absorb organic matter inside the soil. The acidic soil contains low nutrients properties needed for plant uptake. Acidic soil also prevents growth of the plant because the soil contains high toxicity of aluminium (Al) and manganese (Mn) but deficiency in calcium (Ca), magnesium (Mg) and phosphorus (P) (Haynes and Mokolobate, 2001). Moreover, in some area like Selangor, Kelantan, Perlis, Kedah and Perak, limited water source and growing competition for clean water reduced growth performance of banana. Many crop experienced water stress which negatively affect the plant's production. Drastic climate changes in this few years also become major problem since it can affect whole plant growth performance.

For the past few years also, many areas in Malaysia experienced flood which adversely effected to agriculture sector. The flood generally causes the spreading of the soil and water borne diseases. The most commonly occur and devastated disease in banana is Fusarium wilt and Moko disease. Re-emergence of soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (Foc) is one of the major

constraints in banana production. The disease inoculum usually disseminated via contaminated soil on wheels and shoes, contaminated tools, runoff, flooding and irrigation water which eventually infected planting material (Ploetz, 2015). Upon infection to the root tissue, first they invade the xylem vessel, caused necrosis on the corm, restricted the water movement in the vascular bundles and eventually resulted in lethal wilting of the infected plant (Yadeta and Thomma, 2013). Early infection during vegetative stage caused plant to die without bearing fruit.

Currently, enrichment of soil fertility using organic amendments and induction of disease tolerance using plant growth promoting rhizobacteria has been proven to improve soil fertility and increase plant tolerance to disease in several crop plants. However, there is little information about the interactive effect of rhizobacteria with biochar and/or compost, particularly under water stress conditions of banana plants. Because of the relatively large area of Ultisols in the tropics, and thus the high potential for banana cultivation, it is important to develop measures to ameliorate the soil acidity and to reduce disease severity for sustainable banana production. Hence in view of the above problems, the current study was conducted with the following objectives:

1. To determine the optimal rate and effectiveness of enriched biochar on the physicochemical properties of media, growth and physiological status of banana at vegetative stage.
2. To determine the effect of *Bacillus subtilis* on growth, physiological status and biochemical accumulation for suppression of Fusarium wilt disease under water stress condition in enriched biochar amended soil.

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BIODATA OF STUDENT

Siti Norliza Binti Mohd Din was born in 10 February 1993 in Pasir Puteh, Kelantan. She began her primary school in Sekolah Kebangsaan Gong Garu in 2000. Later, she extended her secondary education in Sekolah Menengah Kebangsaan Jeram in 2006 and completed her SPM in 2010. She furthered her study in Foundation of Agriculture Science for one year at Universiti Putra Malaysia and continued with her Bachelor Degree in Agriculture Science. She obtained her Bachelor Degree in 2016 and straightforward enrolled to her Master Degree study majors in Horticulture Science at Department of Crop Science, Faculty of Agriculture, UPM. During her study period, she has participated as poster presenter in Malaysian Society of Plant Physiology Conference (21-23 August 2017) and 6th International Agriculture Student Symposium (4-13 February 2018). She also participated as a member in a community programme of “Empowering human talent of the prison’s community through lifelong learning in banana herbs industrial cultivation opportunity”, a programme under UCTC. She was ambitious to be a lecturer and a generous businesswoman. The author love nature and agriculture. She was hoping that agriculture in Malaysia could be an outstanding field in the world occupied with a new technology and innovation.

LIST OF PUBLICATION

- Norliza, M.S.,** S.S. Zaharah, K. Sijam, M.Z. Aiman Takrim, B. Ali, T. Manickam & J. Tan. (2018). Effect of Biorichar amendment on growth, nutritional properties and biochemical changes of banana (*Musa acuminata*) cv. berangan established in an ultisol soil at vegetative stage. *Journal of Tropical Plant Physiology*, accepted manuscript.
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